



SECTION HEAD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

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per 067907

MEMORANDUM

SUBJECT: Chlorophacinone (Rozol®)

HED Project No. 2-0094

Tox. Chem. No. 211C

FROM: Ray Landolt *6/29/92*
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THRU: Mike Ioannou, Section Head
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J. Ioannou 6/29/92

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Registrant: LiphaTech

Action Requested: In response to the FIFRA 88 reregistration data requirements, a reformat subchronic oral toxicity study in rats (82-1) has been submitted on the technical material for review.

Conclusion: This study (MRID 920180-13) is not acceptable and does not satisfy the guideline data requirement (82-1) for a rodent subchronic oral toxicity study. This study may be upgraded if a NOEL is demonstrated in this study and the data requested in the attached DER is provided.



008566

Reviewed By: Ray Landolt *6/29/92*
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Mike Ioannou *M.I. 6/29/92*
Section I, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

Study Type: 90-day Oral Toxicity- Rat (82-1)

Test Material: Chlorophacinone, Rozol®

Chemical Name: 2-((p-Chlorophenyl)phenylacetyl)-
1,3-indandione

Project No. 2-0094
Tox Chem No. 211C
MRID No. 920180-13
(Reformatted Study)

CAS No. 3691-35-8

Classification: Anticoagulant Rodenticide

Study No.: LCR No. 84.05 LM91 RPP

Date of Study: December 18, 1984

Sponsor: LiphaTech

Testing Facility: Lipha Research Center, Lyon, France

Author: C. Mally, G. Porret-Blanc, and G. Lorgue

Quality Assurance: S. Merle

Report Reformatted by: Ronald L. Baron

Conclusion: Classification of Data - Supplementary

This study does not satisfy the guideline data requirement (82-1) for a 90-day oral toxicity study.

Deficiencies: 1. A No Effect Level was not demonstrated.

Study authors concluded that due "to the total absence of toxicological effect after 77 days treatment" the animals at the 5 ug/kg level (lowest level tested) were sacrificed.

The question remains as to whether an effect would have been apparent at the 5 ug/kg level, after 113 days treatment, when subjected to the same clinical and terminal evaluation as the 10, 20 and 40 ug/kg levels.

Hematology and clinical chemistry parameters were not determined for the 5 ug/kg level.

2. Purity of the test material was not reported.

3. Clinical signs of toxicity were not reported in tabular form.

Conclusion: (con't)

The following effects were reported for the lowest level tested (10 ug/kg) to complete the projected experimental period of 16 weeks.

At the 10 ug/kg level males exhibited an increased body weight gain of 3% accompanied by an increase in food (6%) and water (5%) intake. By comparison females at this level exhibited a decrease in body weight gain of 8% accompanied by a decrease in food (8%) consumption.

Male and female coagulation time was significantly ($p < 0.05$) prolonged at the 10 ug/kg level by 28 and 6%, respectively.

A significant ($p < 0.05$) increase in creatinine (17%), cholesterol (15%), and total protein (4%) accompanied by a decreased in triglyceride (29%) values was reported for females at the 10 ug/kg level.

Male adhenal weights at the 10 ug/kg level were significantly ($p < 0.01$) increased for absolute, relative to body and brain weight by 28, 26 and 29%, respectively.

Experimental Design:

Animals: Ninety male and ninety female 6-week old Sprague Dawley rats weighing between 180 to 186 g for males and 152 to 165g for females were used in this study.

Test material: Chlorophacinone, of unknown purity, was dissolved in corn oil for preparation of the stock solutions and working dilutions. Analyses were within acceptable analytical limits. Controls received corn oil at 5 ml/kg by gavage.

Procedure: Chlorophacinone was administered by gavage at 5 ml/kg 7-days per week to 10 rats/sex at dose levels of 5, 10, 20, 40, 80 and 160 ug/kg b.wt. per day.

<u>Group</u>	<u>Dose ug/kg</u>	<u>Number of Rats</u>		<u>Duration Days</u>
		<u>Males</u>	<u>Females</u>	
T	Control	10	10	113
A	5	10	10	77
B	10	10	10	113
C	20	10	10	113
T ₁	Control	10	10	112
D	40	10	10	112
T ₂	Control	10	10	16
E	80	10	10	16
F	160	10	10	8

The study was initiated with dose levels of 5, 10 and 20 ug/kg b.wt./day, after 5 weeks due to minimal effects, an additional level of 40 ug/kg was initiated. The 5 ug/kg level was terminated after 77 days on study due to minimal toxicity at this level. Subsequently, due to limited mortality at the 40 ug/kg level two additional levels of 80 and 160 ug/kg were initiated.

Each rat was individually identified. The rats were housed 5/sex/cage. Food and water were available ad libitum. Temperature, relative humidity, lighting and air changes were controlled to provide a uniform environment.

Statistics: Significant differences between the control and test groups for body weight, hematology, clinical chemistry and organ weights were analyzed by t-test (Student and Fischer) or by U-test (Mann and Whitney). A comparison of food and water consumption, averages per rat per day, was determined from weekly measurements. "A Bifactorial analysis (time and treatment) was performed".

Methods and Results:

1. Observations:

- a. Gross - All animals were observed twice daily, once before and after treatment, for signs and duration of toxicity. These observations were not reported in tabular form.

The study author reported "The dominant clinical signs that were responsible for death of animals were related to the anticoagulant activity of chlorophacinone".

The following generalized observation was presented not related to the dose administered.

"During a variable time and inversely related to the dose administered (generally between 1 and several (7-10) days), the animals showed some weakness, enfeeblement marked by a lack of energy. This weakness increased until complete immobility (with refusal to get up or to move when incited). At this stage, the hair was often bristled and the back arched. Along with hemorrhages, the following were noted: nosebleeds with quick, spasmodic and difficult breathing. This dyspnea was an indication of pulmonary and/or thoracic hemorrhages which were the most frequently encountered".

- b. Mortality - No deaths were reported at the 5 ug/kg level during the 11 week treatment period. The two deaths (1/10 males and 1/10 females at the 10 ug/kg) level were attributed to intubation error. Males were observed to be more sensitive to the anticoagulant effects of chlorophacinone than females by the clinical signs of toxicity and mortality. At the 160 ug/kg level, males died within 3-days as compared to 4-days for females.

The following table summarizes mortality and time to death.

<u>Dose</u> <u>ug/kg</u>	<u>Death</u>		<u>Time to Death(days)</u>	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
Control	0/30	0/30	-	-
5	0/10	0/10	-	-
10	1/10*	1/10*	10	104
20	4/10	0/10	105-111	-
40	10/10	4/10	29-82	69-111
80	10/10	10/10	7-13	9-16
160	10/10	10/10	5-7	5-8

* death due to intubation error

- c Body weight - All animals were weighed initially, weekly and prior to sacrifice. The dose administered was adjusted for the change in body weight.

Males exhibited an increased body weight gain at the 5, 10 and 20 ug/kg levels by 7, 3 and 9 %, respectively, being significant ($p < 0.05$) at the 20 ug/kg level. Death of 4/10 males was reported at the 20 ug/kg level. By comparison, females at the 5, 10 and 20 ug/kg levels exhibited a decreased body weight gain of 11, 8 and 8 %, respectively with no deaths reported at these levels.

Males at the 40 ug/kg level exhibited a 21% decrease in body weight gain, as compared to the controls, with 10/10 deaths reported between weeks 4 and 11 of the study. By comparison, females at the 40 ug/kg level exhibited a 8% increased body weight gain and no deaths reported.

Both males and females at the 80 ug/kg level exhibited decreased body weight gains of 63 and 60 %, respectively with deaths reported for males (10/10) by week two and for females (10/10) by week three.

Males and females at the 160 ug/kg level exhibited a 100% decrease in body weight gain, being significant ($p < 0.05$) for males, and death during the first week of the study.

The following table summarizes the per cent change in group mean body weight gains as compared to the control values.

		Dose Level (ug/kg)					
		<u>5</u>	<u>10</u>	<u>20</u>	<u>40</u>	<u>80</u>	<u>160</u>
<u>Male</u>	increase	7	3	9*			
	decrease				21	63	100*
<u>Female</u>	increase				8		
	decrease	11	8	8		60	100

* statistically significant ($p < 0.05$)

d. Food consumption was recorded initially then weekly during the study.

Group mean food consumption at the 5, 10 and 20 ug/kg levels increased for males by 14, 6 and 8%, respectively as compared to a decrease of 8% at the 10 and 20 ug/kg levels for female rats. At the 40 ug/kg level male food consumption decreased by 12% as compared to no change for females at this level. Food consumption at the 80 and 160 ug/kg levels decreased by 35 to 37% for males and 21 to 43% for females.

The following table summarizes the percent change observed in group mean food consumption as compared to the control values.

		Dose Level (ug/kg)					
		<u>5</u>	<u>10</u>	<u>20</u>	<u>40</u>	<u>80</u>	<u>160</u>
<u>Male</u>	increase	14	6	8			
	decrease				12	37	35
<u>Female</u>	increase						
	decrease		8	8		43	21

e. Water intake was recorded initially then weekly during the study.

Water intake at the 5, 10 and 20 ug/kg levels increased for males by 18, 5 and 28%, respectively as compared to no change for female rats at these levels. At the 40, 80 and 160 ug/kg levels water intake decreased for males by 9, 22 and 34% respectively and for females by 7, 43 and 18%, respectively.

The following table summarizes the percent change observed in group mean water intake as compared to the control values.

		Dose level (ug/kg)					
		<u>5</u>	<u>10</u>	<u>20</u>	<u>40</u>	<u>80</u>	<u>160</u>
Male	increase	18	5	28			
	decrease				9	22	34
Female	increase						
	decrease				7	43	18

2. Clinical Findings: All animals were fasted overnight prior to collection of samples. At the termination of the study the animals were anesthetized with ether for collection of blood from the retro-orbital sinus. Hematology and clinical chemistry parameters were determined from controls and survivors of the 10, 20 and 40 ug/kg levels, but not for the 5 ug/kg

- a. Hematological parameters examined: The checked (*) parameters are recommended by Subdivision F testing guidelines of November 1989.

* Erythrocyte count	* Leucocyte count
* Hemoglobin	* Differential count
* Hematocrit	* Platelet count
Mean Corpuscular Volume	Quick time (coagulation)
Mean Corpuscular Hemoglobin	Mean Corpuscular Hemoglobin Concentration

Coagulation time was significantly ($p < 0.01$) prolonged for males, as compared to the controls, at the 10 and 20 ug/kg level by 28% and in excess of 100%, respectively. Female coagulation time at the 10 and 20 ug/kg levels was significantly ($p < 0.05$) prolonged by 6% and 11%, respectively. The coagulation time of females at the 40 ug/kg level was prolonged significantly ($p < 0.01$) in excess of 100%.

A significant ($p < 0.01$) increase in female platelet count was reported at the 10 ug/kg level by 32%, 20 ug/kg (not determined) and 40 ug/kg level by 28%.

The significant ($p < 0.01$) increase in hemoglobin (19%), erythrocyte (26%) and hematocrit (13%) values reported for females at the 20 ug/kg level was not observed for females at the 40 ug/kg level.

Mean corpuscular volume decreased significantly ($p < 0.001$) in females by 8% at the 20 ug/kg level, but was comparable to the control values at the 40 ug/kg level.

The following table summarizes the per cent change in group mean hematology parameters as compared to the control values.

	Dose level (ug/kg)		
	<u>10</u>	<u>20</u>	<u>40</u>
Male - coagulation time - prolonged	28**	>100**	
Female - coagulation time - prolonged	6*	11*	>100**
platelet count - increased	32**	-	28**
hemoglobin - increased		19**	
erythrocytes - increased		26**	
hematocrit - increased		13**	
mean corpuscular volume - decreased		8***	

Statistically significant * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

- b. Clinical chemistry parameters examined: The checked (*) parameters are recommended by the Subdivision F testing guidelines of November 1989. The checked (†) are recommended but were not determined.

* Alkaline phosphatase	* Creatinine kinase†	* Potassium
* Aspartate aminotransferase	* Bilirubin	* Chloride
* Lactate dehydrogenase †	* Cholesterol	* Protein, total
* Blood urea nitrogen	* Calcium	* Albumin †
* Glucose	* Phosphorus	Triglycerides
* Creatinine	* Sodium	Magnesium
Alanine aminotransferase		

Female clinical chemistry values at the 10 ug/kg dose were significantly ($p < 0.05$) increased for creatinine by 17%, for cholesterol by 15% and total protein by 4% accompanied by a significant ($p < 0.01$) decrease in triglycerides by 29%.

Female clinical chemistry values at the 20 ug/kg dose were significantly ($p < 0.05$) decreased for glucose by 30%, phosphorus by 13% and magnesium by 11% accompanied by a significant ($p < 0.05$) increase in alanine aminotransferase activity by 28%.

Male clinical chemistry values at the 20 ug/kg level were significantly ($p < 0.01$) increased for bilirubin and triglycerides in excess of 100% and for blood urea nitrogen by 70%.

Clinical chemistry values for females at the 40 ug/kg dose increased significantly ($p < 0.05$) for creatinine by 13%, cholesterol by 27%, triglycerides by 83%, phosphorus by 92%, calcium by 8%, magnesium by 41%, potassium by 88%, and alanine aminotransferase by 89%.

In general, the significant changes in clinical chemistry findings were variable and did not correlate with the dose administered, but are suggestive of liver and kidney injury.

- c. Urinalysis - Urine was collected overnight from the male and female controls, 10 and 20 ug/kg levels and from females of the 40 ug/kg level. The following parameters were examined. The checked (*) parameters are recommended by Subdivision F testing guidelines of November 1989.

* Appearance	* Protein	* Sediment
* Blood	* Bilirubin	* Specific gravity
* Glucose	* Urobilinogen	* Volume
* Ketone bodies	Reducing substances	

No significant changes were reported.

3. Terminal Observations - At the end of the experimental period (16 weeks) the animals were sacrificed with carbon dioxide for macroscopic and microscopic examination. The following tissues were collected for histopathological examination and the checked (x) organs were weighed.

Microscopic examination was limited to tissues from animals that died during and at the conclusion of the study from the 2 control groups (9 males and 10 females), 20 ug/kg (1 male and 1 female), 40 ug/kg (5 males and 7 females), 80 (2 males and 1 female) and 160 ug/kg level (2 males and 2 females). The 10 ug/kg level was not subjected to microscopic examination.

The animals of the 5 ug/kg level were sacrificed on day 78 and the liver weighed. The liver of one male at the 5 ug/kg level showing macroscopic lesion was examined.

The checked (*) parameters are recommended by Subdivision F testing guidelines of November 1989. In addition, organ weights for pituitary, prostate, thymus, thyroid and uterus were recorded. Lung weights were not recorded. The checked (†) tissues are recommended but were not examined.

*	aorta	*	jejunum	*	peripheral nerve †
*	eyes	*	bone marrow	*	X kidneys
*	cecum	*	X liver	*	esophagus
*	colon †	*	X lung	*	X ovaries
*	duodenum	*	lymph node	*	oviduct
*	X brain	*	stomach	*	pancreas
*	skin	*	mammary gland	*	rectum
*	X heart	*	X spleen	*	spinal cord
*	X testes	*	musculature	*	thyroid/parathyroid
*	ileum	*	epididymis †	*	salivary glands
*	trachea	*	X adrenals	*	thymus
*	pituitary	*	uterus	*	urinary bladder
	tongue		vagina		prostate
			diaphragm		sciatic nerve

a. Organ weights

At the 5 ug/kg level significant ($p < 0.01$) increases in absolute and relative liver weights were reported for males by 48 and 34%, respectively and for females by 24 and 28%, respectively.

Male spleen weights at the 20 ug/kg level were significantly ($p < 0.01$) decreased for absolute, relative to body and brain weight by 28, 35 and 29%, respectively.

Female spleen weights at the 20 ug/kg level were significantly ($p < 0.05$) decreased for absolute, relative to body and brain weight by 24, 22 and 30%, respectively. In addition, at the 40 ug/kg level female spleen relative to body weights were significantly ($p < 0.05$) decreased by 16%.

Organ weights (con't)

Male adrenal weights at the 10 ug/kg level were significantly ($p < 0.01$) increased for absolute, relative to body and brain weight by 28, 26 and 29%, respectively.

Male adrenal weights at the 20 ug/kg level were significantly ($p < 0.01$) increased for absolute, relative to body and brain weight by 38, 27 and 36%, respectively. Male absolute and relative adrenal weights were significantly ($p < 0.01$) increased at the 40 ug/kg level by 34 and 92%, respectively.

Female thymus relative to body weights were significantly ($p < 0.05$) increased by 32% at the 10 ug/kg level. Female thymus weights at the 20 ug/kg level were significantly ($p < 0.05$) increased for absolute and relative to body weight by 34 and 43%, respectively. In addition, female thymus relative to body weights were significantly ($p < 0.05$) increased at the 40 ug/kg level by greater than 100% as compared to the control values.

The study authors concluded that "there was no dose-related weight differences (in absolute or relative values) in the examined organs", and "the differences noted were either of no toxicological significance, or were related to body weight loss of some animals in the days preceeding death". This observation is apparent for organ weights recorded for males and females at the 40, 80 and 160 ug/kg levels.

The following table summarizes the percent change in group mean organ weight.

<u>Male</u>	<u>Spleen Weight - Decreased</u>	Dose Level (ug/kg)		
		<u>10</u>	<u>20</u>	<u>40</u>
	absolute		28**	
	relative to body		35**	
	relative to brain		29**	
<u>Female</u>	<u>Spleen Weight - Decreased</u>			
	absolute		24*	
	relative to body		22*	16*
	relative to brain		30*	
<u>Male</u>	<u>Adrenal Weight - Increased</u>			
	absolute	28**	38**	34**
	relative to body	26**	27**	92**
	relative to brain	29**	39**	
Statistically significant, * $p < 0.05$ and ** $p < 0.01$				

Organs weights (con't)

<u>Female</u>	<u>Thymus Weight</u> - Increased	Dose Level (ug/kg)		
		<u>10</u>	<u>20</u>	<u>40</u>
	absolute		34*	
	relative to body	32*	43*	>100*
	relative to brain			
Statistically significant * $p < 0.05$				

b. Macroscopic observations

In general, the incidence of hemorrhagic tissue involved the thymus with hemorrhage observed in the cranial, peritoneal, and thoracic cavities of males and females, accompanied by hemorrhage of the pancreas, pituitary and testes of males.

c. Microscopic observations

Hepatic coagulation necrosis was reported for the one male examined at the 5 ug/kg level.

For those tissues examined at the 40 ug/kg level, hepatic hematopoiesis, centrolobular necrosis and meningeal hemorrhage were observed for males and females accompanied by ovarian hemorrhage for females and interstitial testicular hemorrhage for males at this level.

Lymphoid hyperplasia in the spleen was observed for males at the 40 ug/kg level and in the spleen of those males and females examined at the 80 and 160 ug/kg levels.

Conclusion:

This study does not satisfy the guideline data requirement (82-1) for a 90-day oral toxicity study.

Classification of Data - Supplementary

Deficiencies- 1. A No Effect Level was not demonstrated.

Study authors concluded that due "to the total absence of toxicological effect after 77 days of treatment" the animals at the 5 ug/kg level (lowest level tested) were sacrificed.

The question remains as to whether an effect would have been apparent at the 5 ug/kg level, after 113 days treatment, when subjected to the same clinical and terminal evaluation as the 10, 20 and 40 ug/kg levels.

2. Hematology and clinical chemistry parameters were not determined for the 5 ug/kg level.
3. Purity of the test material was not reported.
4. Clinical signs of toxicity were not reported in tabular form.

The following effects were reported for the lowest level tested (10 ug/kg) to complete the projected experimental period of 16 weeks.

At the 10 ug/kg level males exhibited an increased body weight gain of 3% accompanied by an increase in food (6%) and water intake (5%). By comparison, females at this level exhibited a decrease in body weight gain of 8% accompanied by a decrease in food (8%) consumption.

Male and female coagulation time was significantly ($p < 0.05$) prolonged at the 10 ug/kg level by 28 and 6%, respectively.

A significant ($p < 0.05$) increase in creatinine (17%), cholesterol (15%) and total protein (4%) accompanied by a decrease in triglyceride (29%) values was reported for females at the 10 ug/kg level.

Male adrenal weights at the 10 ug/kg level were significantly ($p < 0.01$) increased for absolute, relative to body and brain weight by 28, 26 and 29%, respectively.

Lab/Study #/Date	Material	EPA Accession No.	Results: LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
<p>day Oral Toxicity - Rat</p> <p>2 Tech LCP No. 84.05</p> <p>91.8PP 12/18/84</p>	<p>Chlorophacinone</p> <p>(purity not reported)</p>	<p>920180-13</p> <p>Reformulated</p>	<p>NOEL was not demonstrated.</p> <p>10 ug/kg Increased b.wt. for males (3%), decreased b.wt. for females (8%).</p> <p>Prolonged coagulation time for males (28%) and females (6%).</p> <p>Increased creatinine (17%), cholesterol (54%)</p> <p>Protein (4%) and decreased triglycerides (29%) for females.</p> <p>Livers tested by gavage 7 days for 16 weeks 16, 20, 40, 80 and 160 ug/kg.</p> <p>adipose { Increased male absolute, relative livers and brain weight by 28, 26 and 29%, respectively.</p>		<p>Supplementary</p>

13544

007320

Chemical:

Chlorophacinone

PC Code:

067707

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